

**STORED COCOA BEANS QUALITY AFFECTED BY  
FERMENTATION AND *EPHESTIA CAUTELLA* WALKER  
(LEPIDOPTERA: PHYCITIDAE) INFESTATION**

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**ABSTRACT**

The effects of fermentation on *Ephestia cautella* population and cocoa beans quality in terms of moisture content, fungal population, the percentage of insect-damaged and mouldy beans, lipid and free fatty acid contents during storage were investigated together with the effects of *£. cautella* infestation on the quality of stored cocoa beans and weight loss.

Fermented and unfermented cocoa beans with initial moisture contents of 7 or 9% were placed in ventilated plastic jars (1kg/jar) and stored for 6 months under room conditions. Seven larvae of *£. cautella* instar IV (2 males and 5 females) were introduced in each jar at the beginning of storage. Untreated jars contained only cocoa beans.

Population of *£. cautella* on fermented cocoa beans with either initial moisture content of 7 or 9% was lower than that on unfermented beans during storage. The population either on fermented or unfermented cocoa beans with initial moisture content of 7% was lower than that of 9%, and the population of all treatments increased during storage. Moisture content of all treatments either on cocoa beans with initial moisture contents of 7 or 9% had the same pattern. The percentage of insect-damaged beans on fermented cocoa beans was lower than that on unfermented cocoa beans after 5 to 6 months of storage. The damaged beans on fermented cocoa after 6 months of storage was not different than on unfermented beans after 4 months of storage. The weight loss either on fermented or unfermented cocoa beans with initial moisture content of 9% was higher than that with initial moisture content of 7%. The weight loss on fermented cocoa beans either with moisture content of 7 or 9% was lower than that on unfermented beans during storage. The weight loss either on fermented or unfermented cocoa beans increased during storage. The percentage of mouldy beans on cocoa infested with *£. cautella* tended to increase during storage, while on beans not infested with the insect it fluctuated during storage. The highest percentage of mouldy beans was on unfermented and infested cocoa beans. Twenty-one fungal species were isolated from all treatments of cocoa beans during storage. The total fungal population on fermented and unfermented beans had the same pattern. The population on fermented cocoa beans was lower than that on unfermented beans. Total lipid content on fermented cocoa beans either infested or not with *£. cautella* having initial moisture content of 7 or 9%, was lower than that of unfermented beans. The content either on fermented or unfermented cocoa beans and either infested or not decreased during storage. Free fatty acid content on cocoa beans infested with *£. cautella* was higher and significantly different than that on not infested. The content for both types increased during storage.

**Key Words:** Cocoa beans / Fermentation / *Ephestia cautella* / Moisture content / Fungal population / Insect-damaged beans / Mouldy beans / Lipid / Free fatty acid.

**INTRODUCTION**

According to the International Cocoa Organization (1996), Indonesia ranks third among the cocoa producing countries of the world after Ivory Coast and Ghana. It has been predicted that the production of Indonesian cocoa beans will

increase by 4.76% per year for the period of 1995-2005. The increase is especially due to the policy which has been determined by the Government of Indonesia through the development and quality improvement of plant material programs (Susila 1996).

Siswoputranto (1997) reported that in Indonesia cocoa is now the fourth most important export commodity after palm oil, rubber and coffee. Compared to West African cocoa beans, Indonesian cocoa beans have an excessive acidic flavor, low chocolate flavor and often certain other objectionable off-flavors. Consequently, they are less desired by cocoa importing countries (Duncan 1990). The imperfections in the fermentation process in particular by smallholders should be improved correctly, if better markets and higher prices for well fermented beans are expected through maintaining the quality of Indonesian (smallholders') cocoa (Siswoputranto 1997).

According to Zaenudin and Wahyudi (1996), insect and mould attacks were the problems of exported cocoa beans derived from smallholders, so that they were subjected to automatic detention. Consequently they should be fumigated, which will need additional expenses. The problem of automatic detention could be minimized by improving the method of postharvest handling from farmer to exporter levels, and thus the quality of Indonesian cocoa beans will also be improved.

With regard to the low quality of cocoa beans, importing country (USA) slapped a fine of 165-180 USD per ton. South Sulawesi exported 120,000-140,000 tons of cocoa beans per year with an export value of 130 million USD (KOMPAS 1996).

Kalshoven (1981) and Wood (1985) reported that *Ephestia cautella* Walker (Lepidoptera: Phycitidae) (tropical warehouse moth) is associated with stored cocoa beans. According to Dharmaputra *et al.* (1999) *E. cautella* was found in some samples of cocoa beans at exporter level in South Sulawesi.

There is an urgent need for Indonesia to improve the quality of smallholders' cocoa beans for having good markets with better prices, and for anticipating whatever changes of markets in this coming era of global economy and international free trade, where severe competitions will be faced by cocoa producing countries, despite deficits of supply which may occur.

The objective of this study was to investigate the effects of fermentation on *E. cautella* population and cocoa beans quality in terms of moisture content, fungal population, the percentage of insect-damaged and mouldy cocoa beans, lipid and free fatty acid content during storage. The effects of *E. cautella* infestation on the quality of stored cocoa beans and weight loss were also analyzed.

## MATERIALS AND METHODS

### Storage of cocoa beans and insect infestation

Fermented and unfermented cocoa beans (bulk type) obtained from Rajamandala Estate Crop (PTPN VIII), Cipatat, Bandung, were used in this study.

Soon after harvest, the pods of cocoa beans were opened and removed. The first category of cocoa beans was fermented using the standard method of the State Estate Crops soon after removing the beans from the pods. After fermentation process, they were washed and sundried until about 7% of moisture content was reached. The second category of cocoa beans was not fermented, but they were washed directly after removing from the pods.

Prior to storage, cocoa beans were fumigated with phosphine at dosage rates of 2g/ton with an exposure period of five days in order to kill any stage of insect that may exist. After fumigation, the moisture content of each cocoa bean was adjusted (7 and 9%). They were then placed in ventilated plastic jars (1 kg/jar) and stored for 6 months under room conditions. Seven larvae of *E. caudella* instar IV (2 males and 5 females) were introduced in each jar at the beginning of storage. Untreated jars contained only cocoa beans. Three replications were used for each treatment.

### Methods of sampling

A sample consisting of the whole content of each jar was taken before storage, and subsequently after 1, 2, 3, 4, 5 and 6 months of storage. Twenty-four samples were taken at each month of storage. Consequently, the number of all experiment units was 168. Insects were separated from cocoa beans using graded sieves. Each cocoa bean sample was then divided several times using a sample divider to obtain working samples for analyzing insect-damaged and mouldy beans, fungal, moisture, lipid and free fatty acid contents.

### Insect and fungal population determinations

Insect population (larva, pupa and adult) per kg of cocoa beans derived from each jar was determined by counting the number of dead and live insects in the outer and inner parts of the beans after separation from the cocoa beans.

Fungal population was determined based on dilution method followed by pour plate method using Dichloran 18% Glycerol Agar (DG18) (Pitt and Hocking 1997). Fungal species was identified using the publications of Samson *et al.* (1996), Pitt and Hocking (1997) as the main references.

### Moisture, lipid and free fatty acid contents analyses

Moisture content (wet weight) of cocoa beans was determined based on SNI 01-2323 (ISC 1998). Two replicates were used for each sample. The beans were ground and dried in the oven at  $103^{\circ} \pm 2^{\circ}\text{C}$  for 16 hours. The moisture content was determined using the following formula:

$$me = (M_1 - M_2) \times \frac{100}{M_1 - M_0}$$

me = moisture content (%)

$M_0$  = the mass, in grams, of the empty dish and its lid  
 $M_1$  = the mass, in grams, of the dish and its lid, and the test portion before drying  
 $M_2$  = the mass, in grams, of the dish and its lid, and the test portion after drying

Total lipid content was determined using Soxhlet extraction method (ISC 1998). The principle of this method is the extraction of free oil from the cocoa bean sample using non polar organic solvent («-hexane) which has been hydrolyzed. Total lipid content was expressed as the percentage of mass and calculated on a dry weight basis, using the following formula:

$$\% \text{ total lipid content} = \frac{(M_1 - M_2) 100}{M_0} \times \frac{100}{100 - mc}$$

$me$  = moisture content of the test sample  
 $M_1$  = the mass, in grams, of the flask and lipid after drying  
 $M_2$  = the mass, in grams, of the dry flask  
 $M_0$  = the mass, in grams, of the test sample

Free fatty acid content was determined using titration method (ISC 1998). Fat obtained from extraction is dissolved in warm ethanol and then titrated using alkali solution (NaOH 0.1N). Free fatty acid was calculated and expressed as the percentage of mass per mass using the following formula:

$$\% \text{ free fatty acid content} = \frac{V \times N}{M} \times \frac{100}{100 - mc}$$

$V$  = the volume, in ml, of NaOH  
 $N$  = the normality of NaOH solution  
 $M$  = the mass, in grams, of cocoa bean lipid  
 $Me$  = moisture content

#### Insect-damaged and mouldy beans, and weight loss determinations

Insect-damaged beans are those the internal parts of which contain dead insects at any stage of development, or which show damage by insects, visible to the naked eye. Mouldy beans are those with fungi (mould) in the inner part. The percentages of insect-damaged and mouldy beans were determined according to SNI 01-2323 (ISC 1998).

The percentage of dry weight loss was determined based on Harris and Linblad (1977) using the following formula :

$$\% \text{ weight loss} = \frac{(U.N_d) - (D.N_u)}{U (N_d = N_u)} \times 100$$

U = weight of undamaged beans  
 Nu = number of undamaged beans  
 D = weight of damaged beans  
 Nd = number of damaged beans

## Experimental data

Experimental data were analyzed using Completely Randomized Factorial Design with 4 factors. The 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> factors were fermentation, initial moisture content, *E. cauttella* infestation and duration of storage, respectively.

## RESULTS AND DISCUSSION

### *E. cauttella* population

The population of *E. cauttella* on fermented cocoa beans with either initial moisture content of 7% or 9% was lower than that on unfermented beans during storage (Figure 1). Preliminary study on the preference test of *E. cauttella* on fermented and unfermented cocoa beans showed that the number of insects found on fermented cocoa beans was lower than that on unfermented cocoa beans (Table 1). It seems that the insects preferred unfermented cocoa beans. According to Wood (1985) the unpleasant smell is caused by high content of acetic acid in fermented cocoa beans.

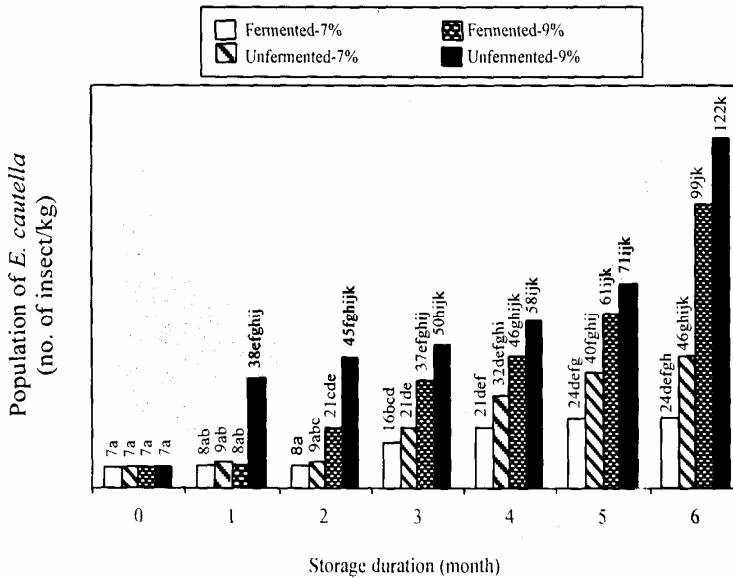


Figure 1. Population of *Ephestia cauttella* on cocoa beans during storage

Table 1. Preference test of *Ephestia cautella* on cocoa beans

| Treatment         | Number of larvae found (insect/replication) |
|-------------------|---|
| Fermented beans   | 1 ± 0.89                                    |
| Unfermented beans | 10 ± 2.37                                   |

The population of *E. cautella* either on fermented or unfermented cocoa beans with " initial moisture content of 7% was lower than that of 9% during storage (Figure 1). The change of moisture content during storage affected the growth and the development of insects. In foodstuff storage, moisture content is one of the important factors related with the growth development of insects (Sinha and Muir 1973; Haines 1991).

Populations of *E. cautella* of all treatments increased during storage. After 6 months of storage, the lowest population was found on fermented cocoa beans with initial moisture content of 7% (24 insects/kg), while the highest was on unfermented cocoa beans with initial moisture content of 9% (122 insects/kg).

### Moisture content

Moisture content of all treatments on cocoa beans with initial moisture contents of 7 or 9% had the same pattern. It decreased until 4 months of storage and then increased after 5 to 6 months of storage (Figure 2). The moisture contents of fermented and unfermented beans with insects tended to decrease until 5 months of storage and increase after 6 months of storage. It was assumed that the change of the moisture content was affected by the insect activity, the presence of fungi and biochemical reactions of the commodities (Sinha and Muir 1973).

### Insect-damaged beans

Insects are the most important cause of deterioration of stored grain ecosystems. *E. cautella* belongs to one of internal feeder insects, because it can move inside of the beans and cause damage to the inner parts of the beans. Deterioration caused by *E. cautella* was determined by the presence of hole on beans, feces, silk webbing, dead bodies and frass.

After 1 month of storage, the percentage of insect-damaged beans on fermented cocoa beans was not different than on unfermented ones. However, the percentage on fermented cocoa beans was lower ( $P < 0.05$ ) than that on unfermented cocoa beans after 5 to 6 months of storage (Figure 3). The population of *E. cautella* on fermented cocoa beans was lower than that on unfermented cocoa beans, consequently the insect-damaged beans on fermented cocoa beans was lower than that on unfermented cocoa beans. Kresnowati (1999) reported that the percentage of damaged beans infested by *Araecerus fasciculatus* on fermented cocoa beans was lower than that on unfermented ones.

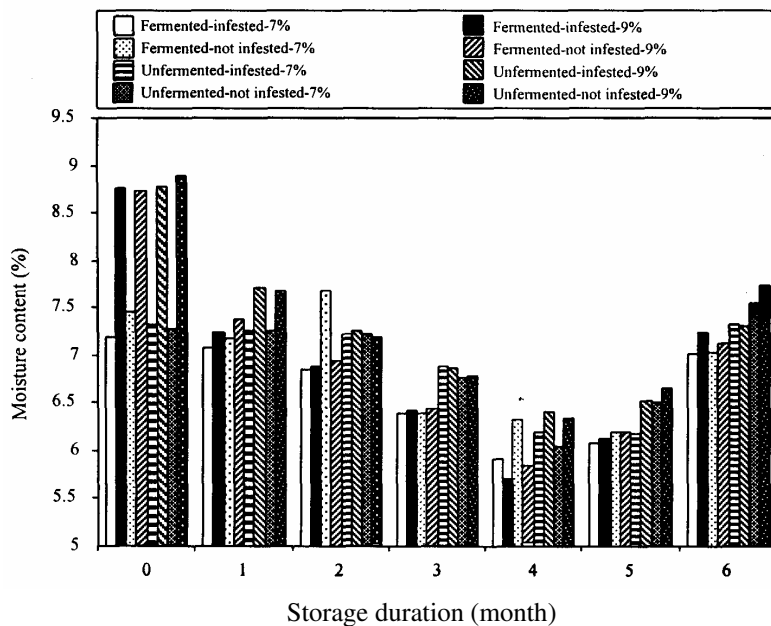


Figure 2. Moisture content of cocoa beans during storage

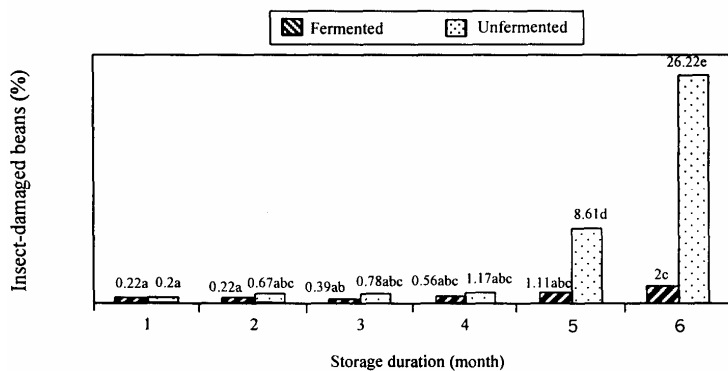


Figure 3. Insect-damaged beans of cocoa during storage (note : SE mean = 1.2554370)

The damaged beans on fermented cocoa after 6 months of storage was not different than on unfermented beans after 4 months of storage. This showed that fermented cocoa beans could be stored for a longer period.

### Weight loss caused by *E. cautella* infestation

The loss agents generally fall into three classes: unavoidable (e.g. weather), human-induced (e.g., contamination, spillage, theft), and pest-induced (e.g. insects, mites, rodents) (Haines 1995).

The weight loss either on fermented or unfermented cocoa beans with initial moisture content of 9% was higher than that with initial moisture content of 7%. Nevertheless, the weight loss of fermented beans with the two initial moisture contents was not significantly different. The highest weight loss was found on unfermented cocoa beans with initial moisture content of 9%. The weight loss on fermented cocoa beans either with moisture content of 7 or 9% was lower than that on unfermented beans during storage (Figure 4). The weight loss either on fermented or unfermented cocoa beans increased during storage (Figure 5). The increase of weight loss was related to the percentage of damaged beans caused by *E. cautella* infestation.

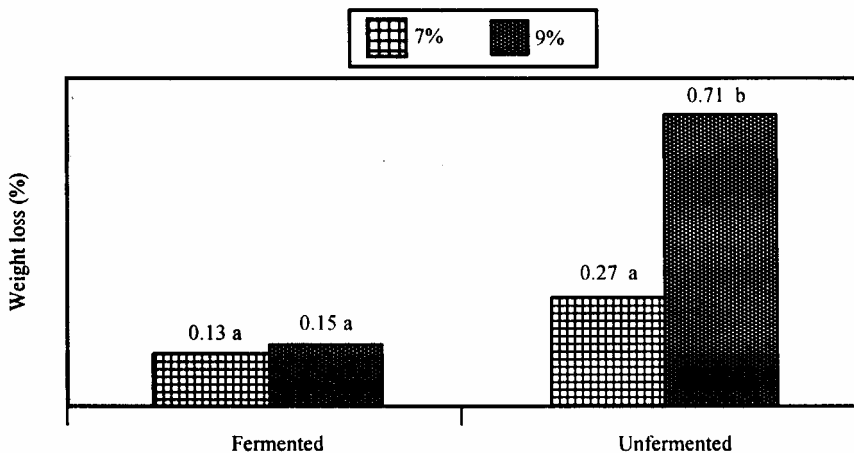


Figure 4. Percentage of weight loss on fermented and unfermented cocoa beans with different initial moisture contents



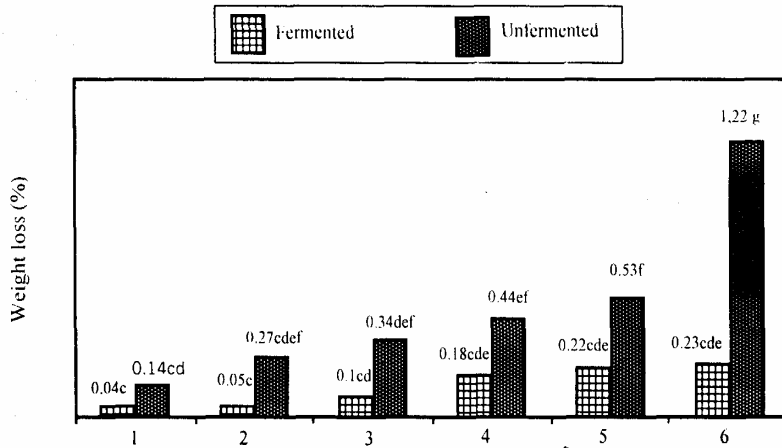


Figure 5. Percentage of weight loss on fermented and unfermented cocoa beans during storage

### Mouldy beans

The highest percentage of mouldy beans was on unfermented and infested cocoa beans (Figure 6). The percentage on fermented cocoa beans increased during storage. The percentage of mouldy unfermented cocoa beans increased after 1 month of storage and decreased until after 3 months of storage, and then increased until after 6 months of storage (Figure 7).

The percentage of mouldy cocoa beans infested with *E. cautella* tended to increase during storage, while the percentage of mouldy cocoa beans that were not infested with the insect fluctuated during storage (Figure 8).

### Species and total fungal population

Twenty-one fungal species were isolated from all treatments of cocoa beans during storage. Fungi isolated from all treatments during storage are shown in Tables 2, 3, 4 and 5. *Cladosporium cladosporioides*, *Eurotium chevalieri* and *Penicillium citrinum* were always isolated from all treatments during storage.

The total fungal population from all treatments during storage was relatively low (Tables 2,3,4 and 5). It was due to the good quality of cocoa beans used in this study. Retnowati *et al.* (2000) reported that the low total fungal population was also found on fermented and unfermented cocoa beans obtained from Rajamandala Estate Crop (PTPN VIII), introduced or not with *A. fasciculatus*.

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Table 2. Species and total fungal population of unfermented cocoa beans, infested with *Ephestia cautella* with initial moisture content of 7 %

| Fungal species                      | Fungal population (cfu/g)<br>Storage duration (month) |           |          |          |          |          |           |          |          |           |           |          |          |          |
|-------------------------------------|---|-----------|----------|----------|----------|----------|-----------|----------|----------|-----------|-----------|----------|----------|----------|
|                                     | 0   |           | 1        |          | 2        |          | 3         |          | 4        |           | 5         |          | 6        |          |
|                                     | I   | NI        | I        | NI       | I        | NI       | I         | NI       | I        | NI        | I         | NI       | I        | NI       |
| <i>Acremonium strictum</i>          | 0   | 0         | 0        | 0        | 0        | 0        | 0         | 0        | 0        | 0         | 0         | 0        | 0        | 1        |
| <i>Aspergillus flavus</i>           | 11  | 0         | 0        | 0        | 0        | 2        | 0         | 0        | 0        | 0         | 0         | 0        | 0        | 0        |
| <i>A. niger</i>                     | 3   | 1         | 0        | 0        | 0        | 0        | 0         | 0        | 0        | 0         | 0         | 0        | 0        | 0        |
| <i>A. versicolor</i>                | 0   | 0         | 0        | 0        | 0        | 0        | 0         | 1        | 1        | 0         | 0         | 0        | 1        | 0        |
| <i>Cladosporium cladosporioides</i> | 0   | 0         | 0        | 1        | 1        | 0        | 8         | 1        | 0        | 9         | 67        | 1        | 0        | 1        |
| <i>Endomyces fibuliger</i>          | 0   | 0         | 0        | 0        | 0        | 0        | 0         | 0        | 1        | 0         | 1         | 3        | 0        | 0        |
| <i>Eurotium chevalieri</i>          | 28  | 14        | 1        | 0        | 0        | 0        | 0         | 1        | 0        | 0         | 0         | 1        | 0        | 1        |
| <i>E. repens</i>                    | 5   | 1         | 0        | 1        | 0        | 0        | 2         | 0        | 1        | 0         | 1         | 0        | 1        | 0        |
| <i>Nigrospora oryzae</i>            | 0   | 0         | 0        | 0        | 0        | 1        | 0         | 0        | 0        | 0         | 1         | 0        | 1        | 0        |
| <i>Penicillium citrinum</i>         | 0   | 3         | 0        | 1        | 0        | 0        | 2         | 0        | 0        | 1         | 0         | 1        | 0        | 0        |
| <i>Pestalotiopsis guepinii</i>      | 0   | 0         | 0        | 0        | 0        | 0        | 0         | 0        | 0        | 0         | 1         | 1        | 0        | 0        |
| <i>Phoma shorgina</i>               | 0   | 0         | 0        | 0        | 0        | 0        | 0         | 0        | 1        | 0         | 0         | 0        | 0        | 0        |
| <i>Syncephalastrum racemosum</i>    | 14  | 0         | 0        | 1        | 0        | 0        | 0         | 0        | 0        | 0         | 0         | 0        | 0        | 0        |
| I<br>NI                             |   |           |          |          |          |          |           |          |          |           |           |          |          |          |
| <b>Total</b>                        | <b>61</b>   | <b>19</b> | <b>1</b> | <b>4</b> | <b>1</b> | <b>3</b> | <b>12</b> | <b>3</b> | <b>4</b> | <b>10</b> | <b>71</b> | <b>7</b> | <b>3</b> | <b>3</b> |

Table 3. Species and total fungal population of fermented cocoa beans, infested and not infested with *Ephestia cautella* with initial moisture content of 7 %

| Fungal species                   | Fungal population (cfu/g)<br>Storage duration (month) |          |          |          |          |          |          |          |          |          |           |          |          |          |
|----------------------------------|---|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|----------|----------|----------|
|                                  | 0   |          | 1        |          | 2        |          | 3        |          | 4        |          | 5         |          | 6        |          |
|                                  | I   | NI       | I        | NI       | I        | NI       | I        | NI       | I        | NI       | I         | NI       | I        | NI       |
| <i>Arthrinium phaeospermum</i>   | 0   | 0        | 1        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0        | 0        | 1        |
| <i>Aspergillus flavus</i>        | 2   | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 1        | 0        | 0        |
| <i>A. versicolor</i>             | 0   | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0        | 0         | 0        | 1        | 0        |
| <i>Chaetomium globosum</i>       | 0   | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0        | 0         | 0        | 0        | 0        |
| <i>Cladosporium caudati</i>      | 0   | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 1        | 0         | 0        | 0        | 0        |
| <i>C. cladosporioides</i>        | 1   | 1        | 0        | 1        | 0        | 1        | 0        | 5        | 2        | 3        | 0         | 1        | 0        | 1        |
| <i>Curvularia lunata</i>         | 0   | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0        | 1        | 0        |
| <i>C. pallescens</i>             | 0   | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 1        | 0        | 0        |
| <i>Endomyces fibuliger</i>       | 0   | 0        | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0         | 0        | 0        | 3        |
| <i>Eurotium chevalieri</i>       | 5   | 1        | 0        | 1        | 0        | 1        | 0        | 0        | 0        | 0        | 14        | 0        | 0        | 0        |
| <i>Hyphopicia burtonii</i>       | 0   | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 4        | 1        | 0         | 0        | 0        | 0        |
| <i>Nigrospora oryzae</i>         | 0   | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0        | 0         | 1        | 0        | 0        |
| <i>Penicillium citrinum</i>      | 0   | 0        | 0        | 1        | 1        | 0        | 0        | 0        | 0        | 1        | 0         | 4        | 3        | 1        |
| <i>Pestalotiopsis guepinii</i>   | 0   | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 1        | 0        | 0        |
| <i>Syncephalastrum racemosum</i> | 2   | 2        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0        | 0        | 0        |
| <i>Ulocladium botrytis</i>       | 0   | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0         | 0        | 0        | 0        |
| <b>Total</b>                     | <b>10</b>   | <b>4</b> | <b>1</b> | <b>3</b> | <b>1</b> | <b>5</b> | <b>1</b> | <b>6</b> | <b>6</b> | <b>5</b> | <b>14</b> | <b>9</b> | <b>5</b> | <b>6</b> |

I = infested

NI = not infested

Table 4. Species and total fungal population of unfermented cocoa beans, infested and not infested with *Ephestia cautella* with initial moisture content of 9 %

| Fungal species                      | Fungal population (cfu/g) |           |          |          |          |          |          |          |           |           |           |           |           |           |
|-------------------------------------|---------------------------|-----------|----------|----------|----------|----------|----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                                     | Storage duration (month)  |           |          |          |          |          |          |          |           |           |           |           |           |           |
|                                     | 0                         |           | 1        |          | 2        |          | 3        |          | 4         |           | 5         |           | 6         |           |
|                                     | I                         | NI        | I        | NI       | I        | NI       | I        | NI       | I         | NI        | I         | NI        | I         | NI        |
| <i>Arthrinium phaeospermum</i>      | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0         | 0         | 0         | 0         | 1         |
| <i>Aspergillus flavus</i>           | 0                         | 9         | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0         | 0         | 0         | 0         | 0         |
| <i>A. niger</i>                     | 1                         | 27        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0         | 0         | 0         | 0         | 0         |
| <i>A. versicolor</i>                | 0                         | 0         | 0        | 0        | 1        | 0        | 0        | 0        | 0         | 0         | 0         | 0         | 2         | 0         |
| <i>Chaetomium globosum</i>          | 0                         | 0         | 0        | 0        | 1        | 0        | 1        | 0        | 0         | 0         | 0         | 1         | 5         | 1         |
| <i>Cladosporium cladosporioides</i> | 0                         | 1         | 2        | 1        | 2        | 0        | 1        | 0        | 13        | 6         | 3         | 2         | 1         | 2         |
| <i>Curvularia lunata</i>            | 0                         | 0         | 0        | 0        | 0        | 0        | 1        | 0        | 0         | 0         | 0         | 0         | 0         | 0         |
| <i>Endomyces fibuliger</i>          | 0                         | 0         | 0        | 0        | 0        | 0        | 1        | 1        | 1         | 0         | 82        | 23        | 0         | 0         |
| <i>Eurotium chevalieri</i>          | 8                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0         | 0         | 1         | 8         | 5         |
| <i>E. repens</i>                    | 1                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0         | 0         | 0         | 0         | 0         |
| <i>Fusarium semitectum</i>          | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 1         | 0         | 0         | 0         | 0         |
| <i>Nigrospora oryzae</i>            | 0                         | 0         | 0        | 0        | 0        | 1        | 1        | 0        | 0         | 1         | 0         | 1         | 0         | 1         |
| <i>Penicillium citrinum</i>         | 0                         | 20        | 0        | 0        | 0        | 0        | 1        | 3        | 0         | 2         | 3         | 1         | 2         | 0         |
| <i>Pestalotiopsis guepinii</i>      | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0         | 0         | 0         | 0         | 1         |
| <i>Syncephalastrum racemosum</i>    | 10                        | 17        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0         | 0         | 0         | 0         | 0         |
| <i>Ulocladium botrytis</i>          | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 1         | 0         | 0         | 0         | 0         | 0         |
| <b>Total</b>                        | <b>20</b>                 | <b>74</b> | <b>2</b> | <b>1</b> | <b>4</b> | <b>1</b> | <b>6</b> | <b>4</b> | <b>14</b> | <b>11</b> | <b>88</b> | <b>29</b> | <b>18</b> | <b>11</b> |

I = Infested

NI = Not Infested

Table 4. Species and total fungal population of fermented cocoa beans, infested and not infested with *Ephestia cautella* with initial moisture content of 9 %

| Fungal species                      | Fungal population (cfu/g) |           |          |          |          |          |          |          |          |           |          |          |          |          |
|-------------------------------------|---------------------------|-----------|----------|----------|----------|----------|----------|----------|----------|-----------|----------|----------|----------|----------|
|                                     | Storage duration (month)  |           |          |          |          |          |          |          |          |           |          |          |          |          |
|                                     | 0                         |           | 1        |          | 2        |          | 3        |          | 4        |           | 5        |          | 6        |          |
|                                     | I                         | NI        | I        | NI       | I        | NI       | I        | NI       | I        | NI        | I        | NI       | I        | NI       |
| <i>Arthrinium phaeospermum</i>      | 0                         | 0         | 1        | 0        | 0        | 0        | 3        | 0        | 0        | 0         | 0        | 0        | 0        | 0        |
| <i>Aspergillus candidus</i>         | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 1         | 0        | 0        | 0        | 0        |
| <i>A. flavus</i>                    | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 1        | 0         | 0        | 0        | 0        | 0        |
| <i>A. niger</i>                     | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 1        | 0        | 1        | 0        |
| <i>A. versicolor</i>                | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0        | 0        | 2        | 1        |
| <i>Chaetomium globosum</i>          | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 1        | 2         | 0        | 0        | 0        | 0        |
| <i>Cladosporium cladosporioides</i> | 0                         | 0         | 0        | 1        | 0        | 0        | 0        | 0        | 0        | 7         | 1        | 1        | 1        | 1        |
| <i>Curvularia pallescens</i>        | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 1        | 2         | 0        | 0        | 0        | 0        |
| <i>Eurotium chevalieri</i>          | 5                         | 14        | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0         | 0        | 0        | 0        | 3        |
| <i>E. repens</i>                    | 1                         | 5         | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0        | 0        | 0        | 0        |
| <i>Nigrospora oryzae</i>            | 0                         | 0         | 0        | 0        | 0        | 2        | 0        | 0        | 0        | 0         | 1        | 1        | 0        | 0        |
| <i>Paecilomyces variotii</i>        | 0                         | 0         | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0         | 0        | 1        | 0        | 0        |
| <i>Penicillium citrinum</i>         | 1                         | 1         | 0        | 0        | 1        | 0        | 0        | 0        | 2        | 1         | 1        | 0        | 0        | 0        |
| <i>Syncephalastrum racemosum</i>    | 1                         | 0         | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0         | 0        | 0        | 0        | 0        |
| <b>Total</b>                        | <b>8</b>                  | <b>20</b> | <b>1</b> | <b>1</b> | <b>1</b> | <b>3</b> | <b>3</b> | <b>1</b> | <b>3</b> | <b>14</b> | <b>4</b> | <b>4</b> | <b>4</b> | <b>5</b> |

I = Infested

NI = Not Infested

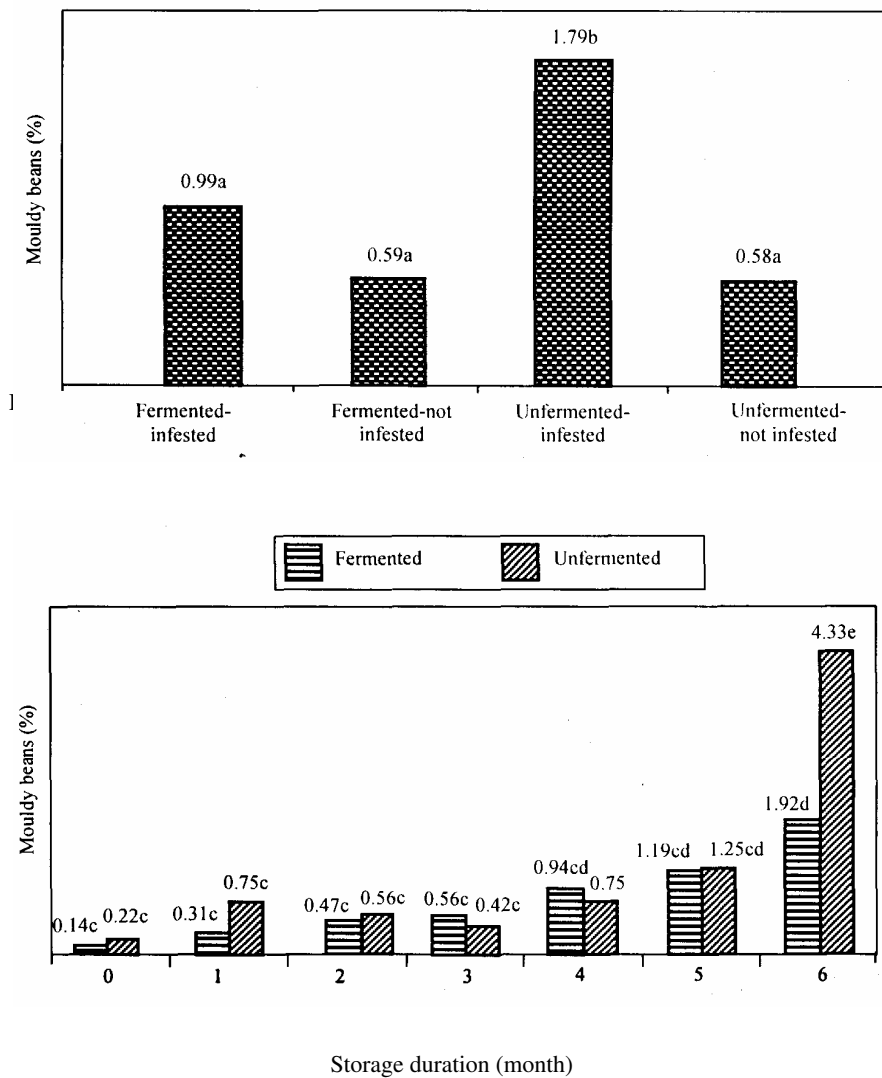


Figure 7. Percentage of mouldy beans on fermented and unfermented cocoa during storage  
(note : SE mean = 0.35747093)

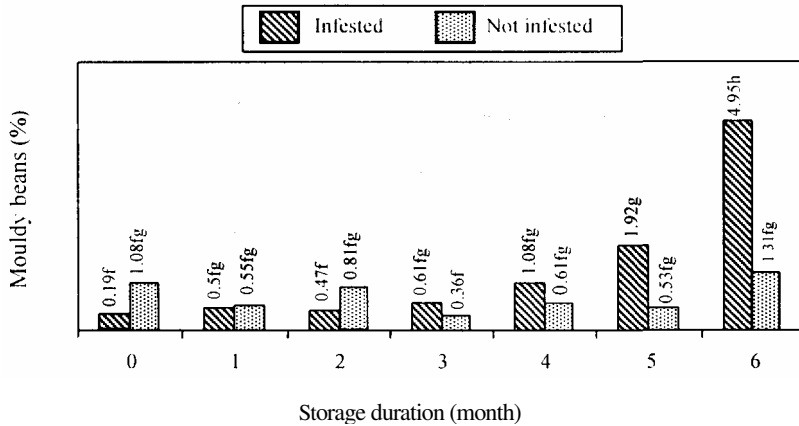


Figure 8. Percentage of mouldy beans on cocoa infested and not infested with *Ephestia caulella* during storage (note: SE mean = 0.35739599)

The total fungal population on fermented and unfermented beans had a similar pattern. It decreased after 1 month of storage and increased after 2 to 5 months of storage, and then decreased again after 6 months of storage. It was assumed that the decrease of total fungal population on unfermented beans after 1 month of storage was due to the interactions among fungi infecting the beans. Nevertheless, the population of fungi on fermented and unfermented beans showed no significant difference ( $P > 0.05$ ) during storage, except on unfermented cocoa beans after 5 months of storage (Figure 9). The fungal population on fermented cocoa beans was lower than that on unfermented beans. Retnowati *et al.* (2000) also reported that total fungal population on fermented cocoa beans was lower than unfermented ones.

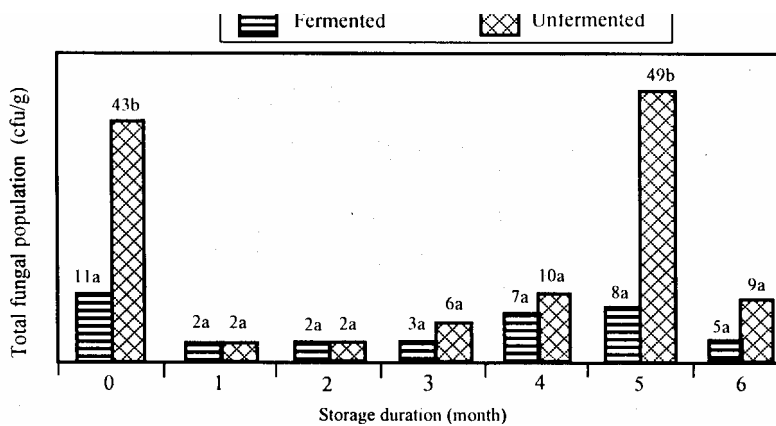


Figure 9. Total fungal population of fermented and unfermented cocoa beans during storage (note : SE mean = 6.59485150)

### Total lipid content

Lipids are triglycerides composed of glycerol and 3-OH group which bind 3 fatty acids (Sherman and Sherman 1989). According to Belitz and Grosch (1987) lipids are important food flavour substances and the most important component in cocoa. Wood (1985) reported that the total lipid content of good cocoa beans quality is between 56 - 58%.

Total lipid content of fermented cocoa beans either infested or not with *E. cautella* having initial moisture contents of 7 or 9% was lower than that on unfermented beans (Figure 10). It was due to the presence of fatty acids produced from fermentation process that stimulate lipid hydrolysis into fatty acids and glycerol. According to Wood (1985) during fermentation process, lactic acid bacteria homo-fermentor transformed glucose into lactic acid. Lactic acid bacteria hetero-fermentor transformed glucose into lactic acid, alcohol, acetic acid and CC>2. Winarno (1991) revealed under certain conditions, such as the presence of acid, lipids are hydrolyzed into fatty acids and glycerol. This study supports the findings of Retnowati *et al.* (2000) that the total lipid content of fermented cocoa beans was lower than that of unfermented beans.

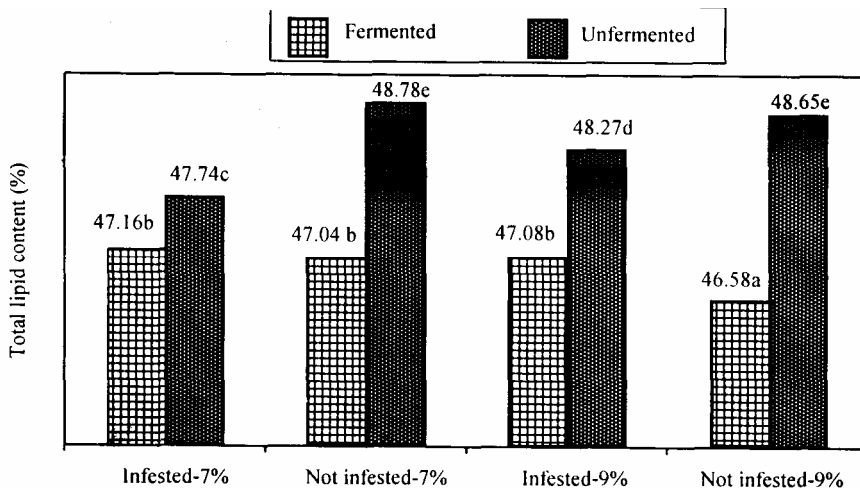


Figure 10. Total lipid content of fermented and unfermented cocoa beans infested and not infested with *Ephesia cautella* having different initial moisture contents (note: SE mean = 0.0648736)

The lipid content of either fermented or unfermented cocoa beans with or without *E. cautella* decreased during storage (Figure 11), due to the transformation of lipids into free fatty acids (Pomeranz 1992). Retnowati *et al.* (2000) also reported that total lipid content decreased during storage.

### Free fatty acid content

The presence of free fatty acids (ffa) gives an indication of the quality of cocoa beans in producing cocoa butter. Cocoa butter with high levels of ffa tend to be soft, have poor crystallization properties, contain off-flavour, and have a poor shelf life (Nickless 1994).

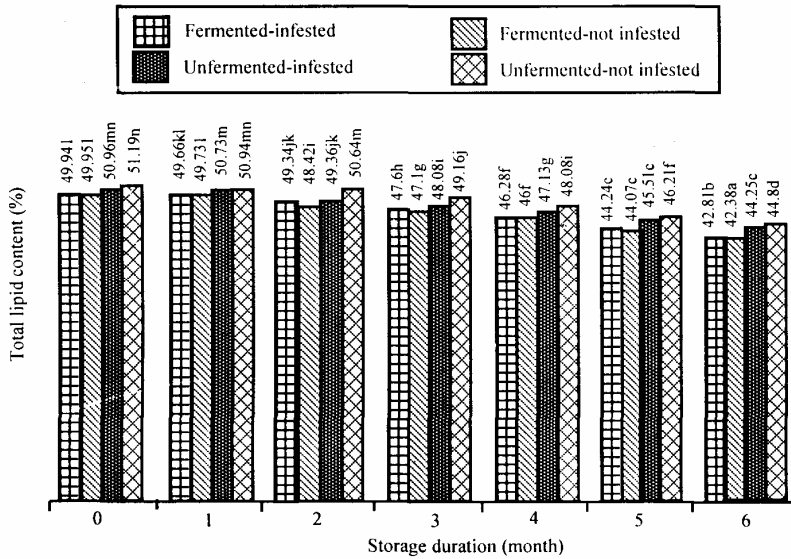


Figure 11. Total lipid content of fermented and unfermented cocoa beans infested and not infested with *Ephestia cautella* during storage (note: SE mean = 0.1213675)

Ffa content of cocoa beans infested with *E. cautella* was significantly ( $P < 0.05$ ) higher than that without insects (Figure 12). The content for both types increased during storage (Figure 13). The content on fermented cocoa beans with initial moisture content of 7% was significantly ( $P < 0.05$ ) lower than that on the beans with initial moisture content of 9%, while the level on unfermented cocoa beans with initial moisture content of 7% was significantly ( $P < 0.05$ ) higher than that on the beans with initial moisture content of 9% (Figure 14). On fermented cocoa beans lipids are easier to be hydrolyzed into ffa, because there are many fatty acids produced from fermentation process. Consequently, ffa on fermented cocoa beans was higher than that on unfermented beans, and it increased during storage.

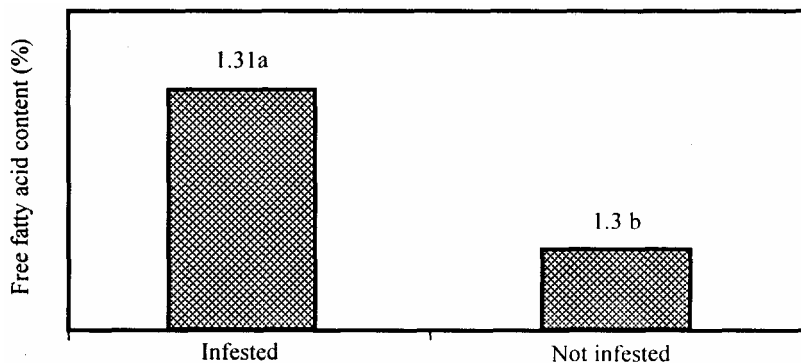


Figure 12. Free fatty acid content of cocoa beans infested and not infested with *Ephestia caoutella* ( note : SE mean = 0.00267261

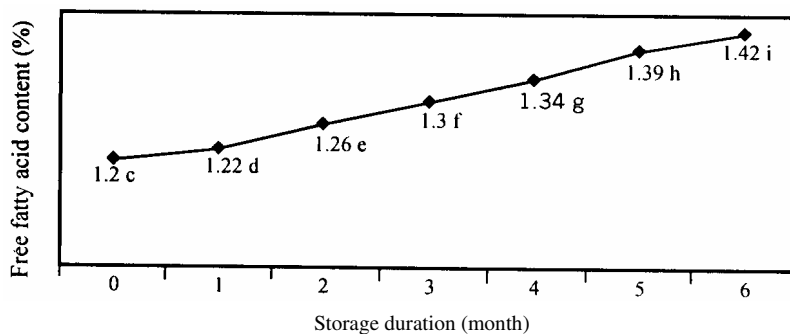


Figure 13. Free fatty acid content of cocoa beans during storage

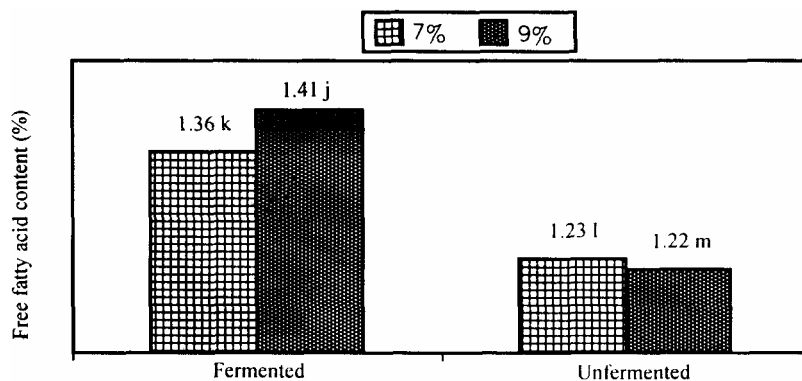


Figure 14. Free fatty content of fermented and unfermented cocoa beans with different initial moisture contents (note : SE mean =0.00377976)



## CONCLUSIONS

In relation to *E. cautella* population and total fungal population during 6 months of storage, the quality of fermented cocoa beans and initial moisture content of  $\pm 7\%$  was better than that of unfermented cocoa beans and initial moisture content of  $\pm 9\%$ .

Infestation of *E. cautella* decreased cocoa beans quality in terms of the percentage of mouldy beans, free fatty acid content, damaged beans and weight loss caused by *E. cautella* during 6 months of storage.

Fermented cocoa beans not infested with *E. cautella* having initial moisture content of 7% could be stored for a longer period than unfermented cocoa beans infested with the insect having initial moisture content of 9%.

Fermentation, insect control and initial moisture content of 7% are the important factors for maintaining the quality of cocoa beans during storage.

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